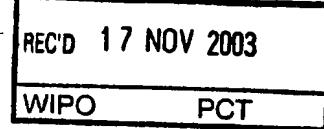


10 / 529772

30 MAR 2005



PCT/NZ03/00225



CERTIFICATE

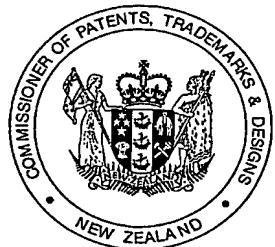
This certificate is issued in support of an application for Patent registration in a country outside New Zealand pursuant to the Patents Act 1953 and the Regulations thereunder.

I hereby certify that annexed is a true copy of the Provisional Specification as filed on 8 October 2002 with an application for Letters Patent number 521851 made by AUCKLAND UNISERVICES LIMITED.

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

Dated 5 November 2003.

Neville Harris
Commissioner of Patents, Trade Marks and Designs



BEST AVAILABLE COPY

330 High Street, Lower Hutt and 17 Toop Street, Seaview
PO Box 30687, Lower Hutt, New Zealand or DX SX 11129, Wellington
Phone: +64 4 560 1600, Fax: +64 4 560 1691, www.iponz.govt.nz

521851



Patents Form No. 4

Our Ref: JC217403

Patents Act 1953

PROVISIONAL SPECIFICATION

NITROANILINE-BASED ALKYLATING AGENTS AND THEIR USE AS
PRODRUGS

We, **Auckland UniServices Limited**, a New Zealand company, of Level 10,
70 Symonds Street, Auckland, New Zealand do hereby declare this invention to
be described in the following statement:

- 1 -

PT0431078

100025746_1

NITROANILINE-BASED ALKYLATING AGENTS AND THEIR USE AS PRODRUGS

The present invention relates to the preparation of nitroaniline-based unsymmetrical
5 mustards, and their use as prodrugs for GDEPT (gene-dependent enzyme-prodrug therapy)
and cell ablation therapy in conjunction with nitroreductase enzymes, as hypoxia-selective
cytotoxins, and as anticancer agents.

BACKGROUND TO THE INVENTION

10

The use of tumour-selective prodrugs (relatively inactive compounds that can be selectively
converted to more active compounds *in vivo*) is a valuable concept in cancer therapy.

15 For example a prodrug may be converted into an anti-tumour agent under the influence of
an enzyme that is linkable to a monoclonal antibody that will bind to a tumour associated
antigen. The combination of such a prodrug with such an enzyme monoclonal/antibody
conjugate represents a very powerful clinical agent. This approach to cancer therapy, often
referred to as "antibody directed enzyme/prodrug therapy" (ADEPT), is disclosed in
W088/07378.

20

A further therapeutic approach termed "virus-directed enzyme prodrug therapy" (VDEPT)
has been proposed as a method for treating tumour cells in patients using prodrugs.

25 Tumour cells are targeted with a viral vector carrying a gene encoding an enzyme capable
of activating a prodrug. The gene may be transcriptionally regulated by tissue specific
promoter or enhancer sequences. The viral vector enters tumour cells and expresses the
enzyme, in order that a prodrug is converted to an active drug within the tumour cells
(Huber et al., *Proc. Natl. Acad. Sci. USA* (1991) 88, 8039). Alternatively, non-viral
methods for the delivery of genes have been used. Such methods include calcium
phosphate co-precipitation, microinjection, liposomes, direct DNA uptake, and receptor-
30 mediated DNA transfer. These are reviewed in Morgan & French, *Annu. Rev. Biochem.*,
1993, 62; 191. The term "GDEPT" (gene-directed enzyme prodrug therapy) is used to
include both viral and non-viral delivery systems.

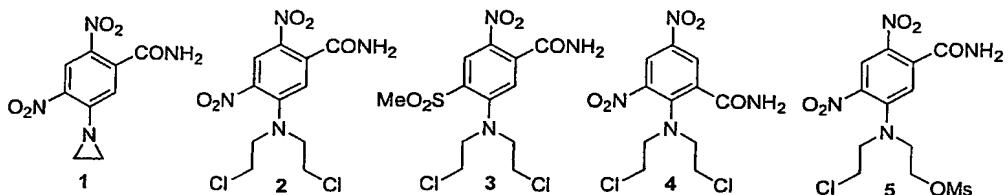
4-Nitroaromatic compounds are reduced by both mammalian and bacterial flavoprotein enzymes, which effect stepwise addition of up to six electrons. The major enzymic metabolite is usually the 4-electron species (hydroxylamine).

5 The present invention relates to novel nitroaniline-based unsymmetrical mustards having cytotoxic activity, to methods of preparing the novel compounds, and to the use of these compounds as prodrugs for GDEPT and for cell ablation therapy in conjunction with nitroreductase enzymes (particularly the nitro reductases encoded by the nfsB gene of *E. coli* or by *Clostridia* species), as hypoxia-selective cytotoxins, and as anticancer agents.

10

Both dinitrobenzamide aziridines (e.g., 1) [Knox et al., *Cancer Met. Rev.*, 1993, 12, 195] and nitro- and dinitrobenzamide mustards (e.g., 2-4) [Friedlos et al., *J. Med. Chem.*, 1997, 40, 1270] have been reported as substrates for the aerobic *E. coli* nitroreductase (NTR), and as specific prodrugs for GDEPT in conjunction with NTR.

15



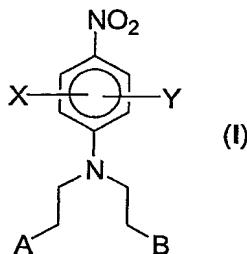
Unsymmetrical (chloro-mesylate) mustards have been reported [e.g., Marais et al., *Cancer Res.* 1996, 56, 4735], including the dinitro analogue 5 [Friedlos et al., *J. Med Chem.* 1997, 40, 1270], which was described as not sufficiently potent for a full biological evaluation to be conducted.

It is therefore an object of the invention to provide a series of unsymmetrical mustards, methods for preparing the unsymmetrical mustards that are suitable for use as prodrugs for 25 GDEPT (gene-dependent enzyme-prodrug therapy) and cell ablation therapy in conjunction with nitroreductase enzymes, as hypoxia-selective cytotoxins, and as anticancer agents or to at least provide the public with a useful alternative.

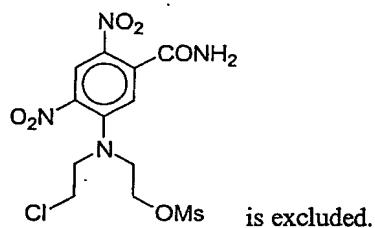
SUMAMRY OF THE INVENTION

In a first aspect, the present invention provides a nitroaniline-based unsymmetrical mustard represented by the general formula (I);

5

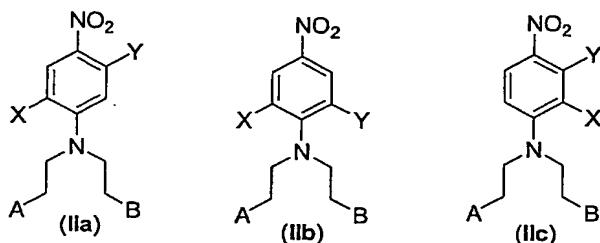


wherein X represents one of the groups NO_2 , CN , or SO_2R^1 , where R^1 represents a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and
 10 wherein when R^1 represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;
 Y represents one of the groups OR^2 , NHCOR^2 , $\text{CONR}^2\text{CO}_2\text{R}^3$, CONR^2 morpholide, CONHR^2 , CONR^2R^3 , CONHOR^2 , $\text{CONHSO}_2\text{R}^2$, SO_2NH_2 , SO_2NHR^2 or $\text{SO}_2\text{NR}^2\text{R}^3$ wherein each R^2 and R^3 independently represent a H, C_{1-6} -lower alkyl optionally substituted with one
 15 or more hydroxy and/or one or more amino groups; and
 A and B each independently represent halogen, OSO_2R^4 , OSO_2NH_2 , OSO_2NHR^4 or $\text{OSO}_2\text{NR}^4\text{R}^5$, wherein each R^4 and R^5 independently represent a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide derivative of the tertiary
 20 amine is further included;
 and pharmaceutically acceptable derivatives and salts thereof;
 with the proviso
 (i) that $\text{A} \neq \text{B}$ and
 (ii) that



In a preferred embodiment the nitroaniline-based unsymmetrical mustard is selected from a compound represented by one of formulae (IIIa-IIIc)

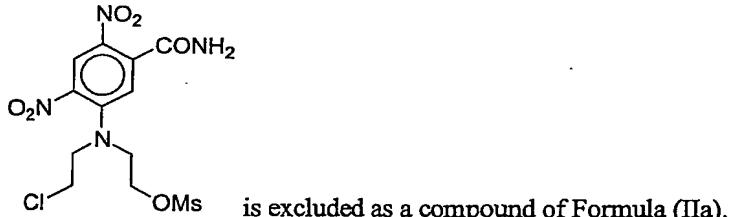
5



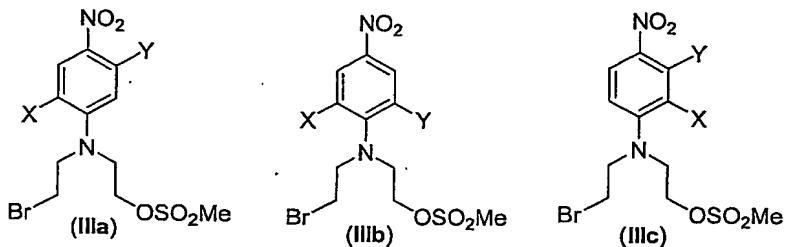
wherein X, Y, A and B are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

10 with the proviso

- (i) that A \neq B and
- (iii) that



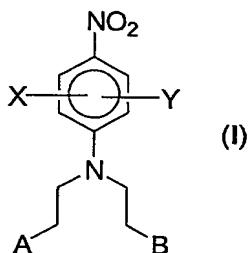
15 In a more preferred embodiment the nitroaniline-based unsymmetrical mustard is selected from a compound represented by one of formulae (IIIa-IIIc)



wherein X, Y, are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof.

5

In a second aspect of the invention there is provided a method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula (I);



10

wherein X represents one of the groups NO_2 , CN , or SO_2R^1 , where R^1 represents a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R^1 represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

15 Y represents one of the groups OR^2 , NHCOR^2 , $\text{CONR}^2\text{CO}_2\text{R}^3$, CONR^2 morpholide, CONHR^2 , CONR^2R^3 , CONHOR^2 , $\text{CONHSO}_2\text{R}^2$, SO_2NH_2 , SO_2NHR^2 or $\text{SO}_2\text{NR}^2\text{R}^3$ wherein each R^2 and R^3 independently represent a H, C₁₋₆-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

A and B each independently represent halogen, OSO_2R^4 , OSO_2NH_2 , OSO_2NHR^4 or

20 $\text{OSO}_2\text{NR}^4\text{R}^5$, wherein each R^4 and R^5 independently represent a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

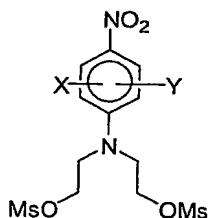
and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

(i) that $A \neq B$

the method including the step of

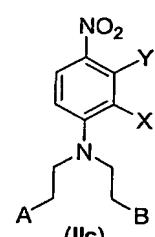
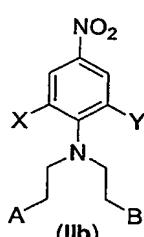
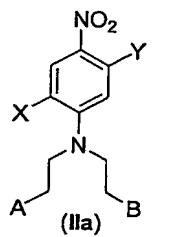
5 (i) reacting a compound of



with an amount of an alkali metal halide in a polar solvent to give an unsymmetrical halo-mesylate compound.

10

In a preferred embodiment the method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula represented by one of formulae (IIIa-IIIc)



15

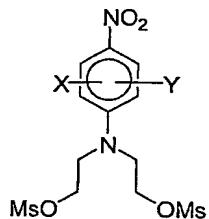
wherein X, Y, A and B are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

(i) that $A \neq B$ and

20 the method including the step of

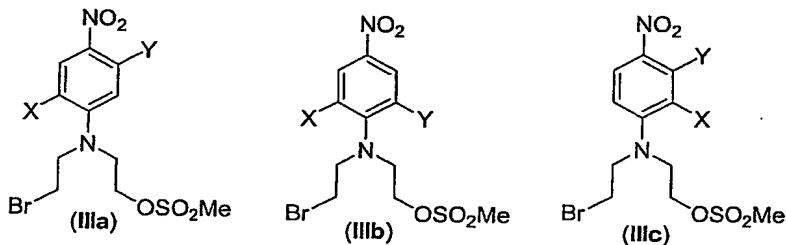
(i) reacting a compound of



with an amount of an alkali metal halide or mesylate halide in a polar solvent to give a unsymmetrical halo-mesylate compound.

5

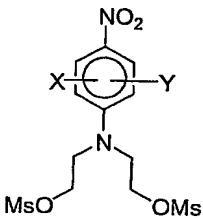
In a more preferred embodiment the method of preparing a nitroaniline-based unsymmetrical mustard represented by one of formulae (IIIa-IIIc)



10

wherein X, Y, are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof; the method including the step of

(ii) reacting a compound of



15

with an amount of LiBr in a polar solvent to give a bromo mesylate of one of formulae (IIIa-IIIc).

20 It is preferred in the methods defined above that the polar solvent is selected from acetonitrile, dimethylformamide, ethyl acetate, triethylamine, acetone and mixtures thereof.

It is preferred in the methods defined above that the alkali metal halide is selected from one or more of the following; LiCl, LiBr, NaI, NaBr,

5 In a third aspect there is provided a compound of formula (I) obtained by any one of the preparative methods defined above.

In a fourth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one

10 nitroreductase enzyme, as hypoxia-selective cytotoxins, including the step of administering a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa-IIc and IIIa-c as defined above or a mixture thereof in a "therapeutically effective amount" to tumour cells in a subject.

15 Preferably, the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

In a fifth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one

20 nitroreductase enzyme, as an anticancer agent including the step of administering a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa-IIc and IIIa-c as defined above or a mixture thereof in a "therapeutically effective amount" to target tumour cells in a subject.

25 Preferably the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

In a sixth aspect of the present invention, there is provided a method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of

30 administering a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa-IIc and IIIa-c as defined above or a mixture thereof in a "therapeutically effective amount" to ablate tumour cells in tissue in a subject, wherein said tissue expresses the at least one nitroreductase enzyme.

Preferably the nitroreductase enzyme is encoded for by the nfsB gene of either *E.Coli* or by *Clostridia* species.

Preferably, the cell ablation therapy provides a substantially minimal bystander effect.

5

In a seventh aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of formula I or a compound of formulae Ia-c, IIa-c, IIIa-c or a mixture thereof, and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

10

The pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser should preferably be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, such as cutaneous, subcutaneous, or 15 intravenous. It is to be appreciated that these factors could be readily determined by someone skilled in the art without undue experimentation.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid 20 pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as gelatin.

25

For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has a suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride 30 injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers antioxidants and/or other additives may be included as required.

In an eighth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a

compound of Formulae Ia-Ic, IIa-IIc and IIIa-c as defined above, for use in GDEPT to target cancer cells in a subject in need thereof.

In a ninth aspect of the present invention there is provided, the use in the manufacture of a
5 medicament of an effective amount of a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa-IIc and IIIa-c as defined above, for use in cell ablation therapy to target cancer cells in a subject in need thereof.

While the compounds of the present invention will typically be used to target tumour cells or
10 tumour tissues in human subjects, they may be used to target tumour cells or tissues in other warm blooded animal subjects such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

As used throughout the specification the term "therapeutically effective amount", is to be
15 understood as an amount of a compound of Formula I as defined above or a compound of any one of compounds Ia-c, IIa-c and IIIa-c as defined above or a mixture thereof that is sufficient to show benefit to a subject with cancer cells. The actual amount, rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment is within the responsibility of general practitioners and other
20 medical doctors.

It is to be understood that the compounds of the invention as defined above may be administered alone or in combination with other treatments, especially radiotherapy, either simultaneously or sequentially dependent upon the condition to be treated.

25 As used throughout the specification the pharmaceutically acceptable derivatives and salts thereof include acid derived salts formed from hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic, methanesulfonic, isethionic acids and the like and base derived salts formed from sodium and potassium
30 carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine and the like.

The technique of cell ablation therapy, would be known to someone skilled in the art. This therapy can be used to selectively ablate specified target cells or tissue through specific

enzymatic expression of a nitroreductase for example, that is specifically expressed by the tissue and which can then be employed to active a prodrug into an active metabolite to ablate the specified target cells or tissue. (Gusterson *et al. Endocrine Related Cancer*, 1997, 4, 67-74.)

5

The expression "substantially minimal bystander effect" is to be understood as meaning that the killing of adjoining non-targeted tumour cells is minimal as a result of diffusion between the targeted tumour cells and non-targeted tumour cells of an activated metabolite that arises from the enzymatic activation of a compound of Formula I as defined above or a compound 10 of any one of compounds Ia-c, IIa-c and IIIa-c as defined above or a mixture thereof.

D

Further aspects of the present invention will become apparent from the following description given by way of example only and with reference to the accompanying synthetic schemes.

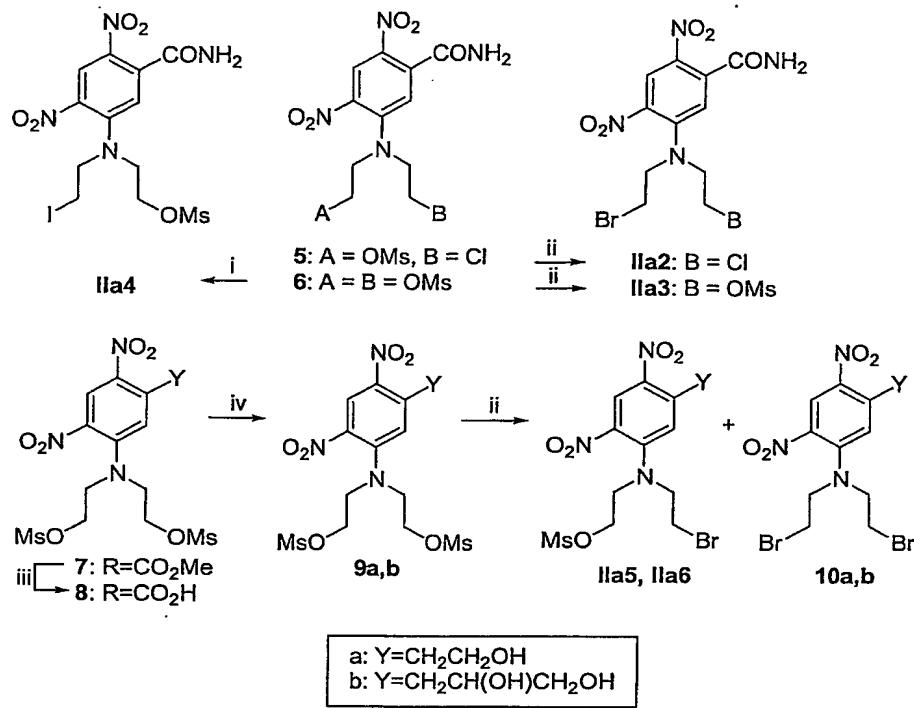
15

DETAILED DESCRIPTION OF THE INVENTION

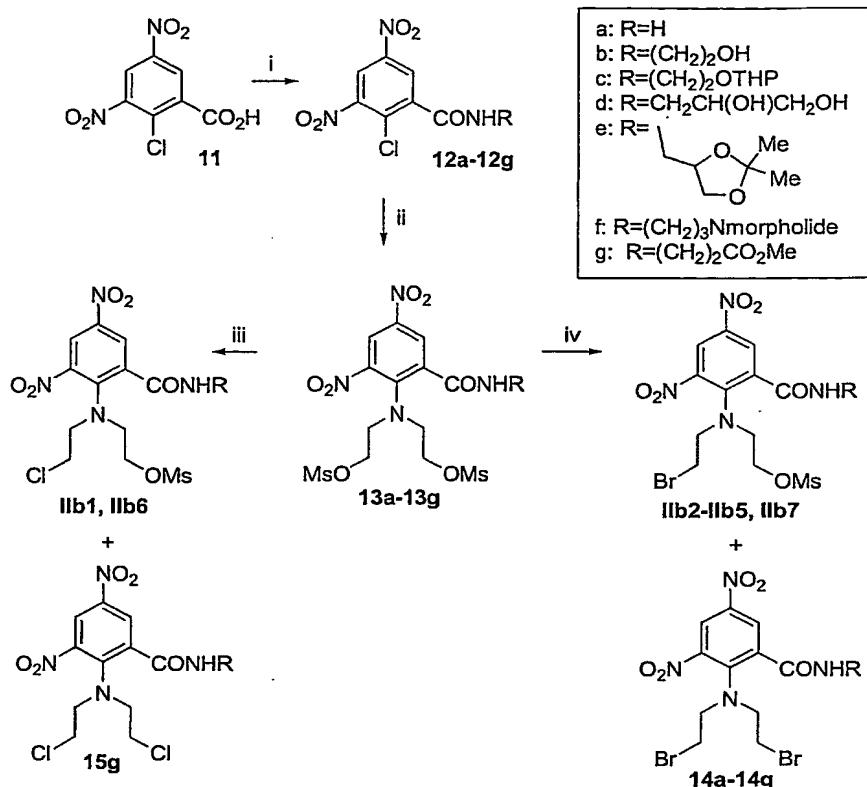
The compounds of formula (I) and the acid addition salts and N-oxides thereof may be prepared by the processes outlined in Schemes 1-3, examples of which are found in Examples 20 A-C.

D

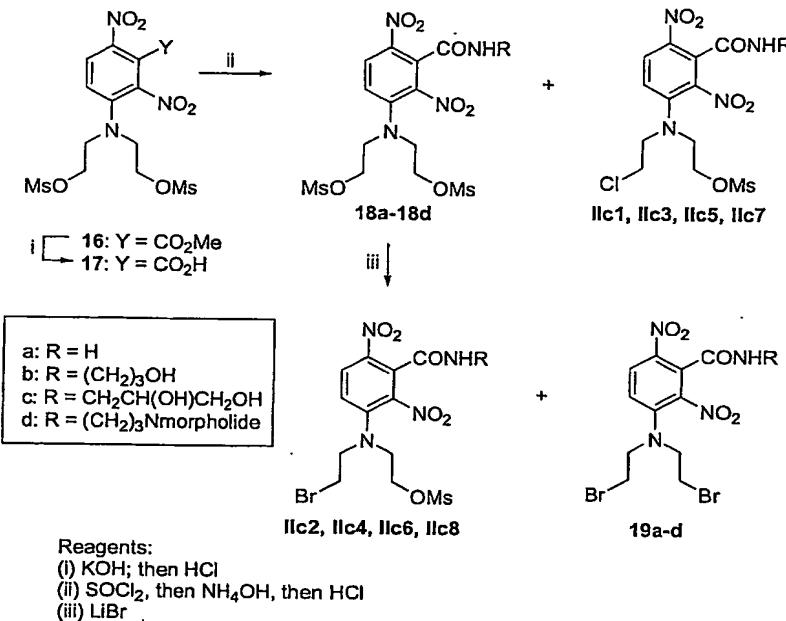
Scheme 1.



Scheme 2.



Scheme 3.



5 In Schemes 1-3, the key reaction is reaction of the dimesylates 6, 9, 13a-13g and 18a-18d with strictly controlled amounts of LiBr or NaI in a polar solvent like DMF or MeCN to give the unsymmetrical bromo- and iodo-mesylate mustards. The method can also be adapted to reaction of the known chloromesylate (5) to give the unsymmetrical chloro/bromo mustard IIa2. While this reaction gives varying amounts of the corresponding bis(bromo) or bis(iodo) compounds as well, these can be easily separated by chromatography to give the pure unsymmetrical mustards.

10

The following Table 1 sets out physicochemical data for 19 compounds within the general formula I, representative of it, and preparable by the processes of the invention.

15

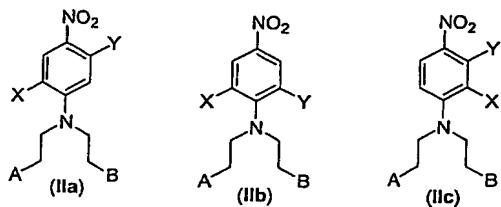


Table 1

No	Y	X	A	B	PRIOR ART COMPOUND		
5	CONH ₂	NO ₂	Cl	OMs	[Friedlos et al., <i>J. Med Chem.</i> 1997, 40, 1270]		

Examples of formula IIa

No	Y	X	A	B	mp (°C)	formula	analyses
IIa2	CONH ₂	NO ₂	Cl	Br	153	C ₁₁ H ₁₂ BrClN ₄ O ₅	C,H,N,Cl
IIa3	CONH ₂	NO ₂	Br	OMs	160-161	C ₁₂ H ₁₅ BrN ₄ O ₈ S	C,H,N,Br
IIa4	CONH ₂	NO ₂	I	OMs	160	C ₁₂ H ₁₅ IN ₄ O ₈ S	C,H,N,I
IIa5	CONHCH ₂ CH ₂ OH	NO ₂	Br	OMs	102-104	C ₁₄ H ₁₉ Br ₂ N ₄ O ₉ S	C,H,N,Br
IIa6	CONHCH ₂ CH(OH)-CH ₂ OH	NO ₂	Br	OMs	117-118	C ₁₅ H ₂₁ BrN ₄ O ₁₀ S	C,H,N,Cl

Examples of formula IIb

No	Y	X	A	B	mp (°C)	formula	analyses
IIb1	CONH ₂	NO ₂	Cl	OMs	155-157	C ₁₂ H ₁₅ ClN ₄ O ₈ S	C,H,N,Cl
IIb2	CONH ₂	NO ₂	Br	OMs	153-154	C ₁₂ H ₁₅ BrN ₄ O ₈ S	C,H,N,Br
IIb3	CONH(CH ₂) ₂ OH	NO ₂	Br	OMs		HRMS	
IIb4	CONHCH ₂ CH(OH)-CH ₂ OH	NO ₂	Br	OMs		HRMS	C,H,N,Br
IIb5	CONH(CH ₂) ₃ Nmorph	NO ₂	Br	OMs		HRMS	
IIb6	CONH ₂ (CH ₂) ₂ CO ₂ Me	NO ₂	Cl	OMs		HRMS	
IIb7	CONH(CH ₂) ₂ CO ₂ Me	NO ₂	Br	OMs		HRMS	

Examples of formula IIc

No	Y	X	A	B	mp (°C)	formula	analyses
IIc1	CONH ₂	NO ₂	Cl	OMs	134-136	C ₁₂ H ₁₅ ClN ₄ O ₈ S	C,H,N,S
IIc2	CONH ₂	NO ₂	Br	OMs	143-145	C ₁₂ H ₁₅ BrN ₄ O ₈ S	C,H,N,Br
IIc3	CONH(CH ₂) ₃ OH	NO ₂	Cl	OMs	104-109	C ₁₅ H ₂₁ ClN ₄ O ₉ S	C,H,N,Cl
IIc4	CONH(CH ₂) ₃ OH	NO ₂	Br	OMs	115-117	C ₁₅ H ₂₁ BrN ₄ O ₉ S	C,H,N
IIc5	CONHCH ₂ CH(OH)CH ₂ OH	NO ₂	Cl	OMs	100-105	C ₁₅ H ₂₁ ClN ₄ O ₁₀ S	C,H,N,Cl
IIc6	CONH ₂ CH ₂ CH(OH)CH ₂ OH	NO ₂	Br	OMs	108-110	C ₁₅ H ₂₁ BrN ₄ O ₁₀ S	C,H,N,Br
IIc7	CONH(CH ₂) ₃ Nmorph	NO ₂	Cl	OMs	113-116	HRMS	
IIc8	CONH(CH ₂) ₃ Nmorph	NO ₂	Br	OMs	114-117	HRMS	

The following Examples A-C illustrate the preparation of compounds representative of the general formula (I).

5 **Example A : Preparation of analogues of class IIa by the method outlined in Scheme 1.**

5-[(2-Bromoethyl)(2-chloroethyl)amino]-2,4-dinitrobenzamide (**IIa2**). A mixture of 2-[5-(aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate (**5**) [Friedlos et al., *J. Med. Chem.* 1997, 40, 1270] (0.91 g, 2.2 mmol) and LiBr (0.21 g, 2.4 mmol) in anhydrous MeCN (25 mL) was stirred under reflux for 1.5 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (3:2) to give a crude product contaminated with the corresponding dibromo mustard. Purification by multiple recrystallisations from EtOAc/I-Pr₂O gave **IIa2** (595 mg, 68%): mp 153 °C; ¹H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.52 (s, 1 H, H-3), 8.17 & 7.82 (2 x s, 2 H, CONH₂), 7.43 (s, 1 H, H-6), 3.82 (t, $J = 5.8$ Hz, 2 H, CH_2Cl), 3.77-3.63 (m, 6 H, N(CH₂-)CH₂CH₂Br). Anal. Calc for $\text{C}_{11}\text{H}_{12}\text{BrClN}_4\text{O}_5$: C, 33.4; H, 3.1; N, 14.2; Cl, 9.6. Found: C, 33.4; H, 3.0; N, 14.1; Cl, 8.9%.

2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (**IIa3**). A mixture of 2-(5-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**6**) [Friedlos et al., *J. Med Chem.*, 1997, 40, 1270] (1.60 g, 3.4 mmol) and LiBr (356 mg, 4.1 mmol) in anhydrous MeCN (30 mL) was stirred under reflux for 1 h. The mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel. Elution with EtOAc/CH₂Cl₂ (11:9) gave the dibromo mustard, while further elution with EtOAc/CH₂Cl₂ (3:1) gave **IIa3** (0.61 g, 39%): mp (EtOAc/I-Pr₂O) 160-161 °C; ¹H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.53 (s, 1 H, H-3), 8.14 & 7.83 (2 x s, 2 H, CONH₂), 7.46 (s, 1 H, H-6), 4.33 (t, $J = 5.1$ Hz, 2 H, CH_2O), 3.74 (t, $J = 5.1$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{O}$), 3.70 (br s, 4 H, $\text{CH}_2\text{CH}_2\text{Br}$), 3.14 (s, 3 H, CH_3). Anal. Calcd for $\text{C}_{12}\text{H}_{15}\text{BrN}_4\text{O}_8\text{S}$: C, 31.7; H, 3.3; N, 12.3; Br, 17.6. Found: C, 32.0; H, 3.4; N, 12.2; Br, 17.7%.

2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (**IIa4**). A mixture of **6** (1.12 g, 2.38 mmol) and NaI (0.46 g, 3.07 mmol) in anhydrous MeCN (20 mL)

was stirred at reflux for 1 h. The mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel. Elution with EtOAc/CH₂Cl₂ (1:1) gave the diiodo mustard, while further elution with EtOAc/CH₂Cl₂ (3:1) gave IIa4 (0.49 g, 41%): mp (Me₂CO/EtOAc/i-Pr₂O) 160 °C; ¹H NMR [(CD₃)₂SO] δ 8.52 (s, 1 H, H-3), 8.14 & 7.83 (2 x s, 2 H, NH₂), 7.44 (s, 1 H, H-6), 4.33 (t, J = 5.1 Hz, 2 H, CH₂O), 3.73 (t, J = 5.1 Hz, 2 H, CH₂CH₂O), 3.65 (t, J = 6.9 Hz, 2 H, CH₂CH₂I), 3.40 (t, J = 6.9 Hz, 2 H, CH₂I), 3.13 (s, 3 H, CH₃). Anal. Calcd for C₁₂H₁₅IN₄O₈S: C, 28.7; H, 3.0; N, 11.2; I, 25.3. Found: C, 29.4; H, 3.0; N, 11.0; I, 25.0%.

10 2-((2-Bromoethyl)5-[(2-hydroxyethyl)amino]carbonyl)-2,4-dinitroanilino)ethyl
methanesulfonate (IIa5). A stirred solution of methyl 5-[bis(2-hydroxyethyl)amino]-2,4-
dinitrobenzoate [Palmer et al., *J. Med. Chem.* 1994, 37, 2175] (5.50 g, 16.7 mmol) and Et₃N
(5.82 mL, 41.8 mmol) in dry CH₂Cl₂ (50 mL) was treated dropwise at 0 °C with MsCl (3.14
mL, 40.0 mmol). After 30 min, 10% aqueous KHCO₃ (100 mL) was added, and the mixture
15 was stirred for a further 30 min at 0 °C and then diluted with pet. ether (500 mL). The
precipitated product was collected and washed with water and iPr₂O to give methyl 5-(bis{2-
[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoate (7) (7.44 g, 92%): mp (CH₂Cl₂/pet.
ether) 157-158 °C; ¹H NMR [(CD₃)₂SO] δ 8.62 (s, 1 H, H-3), 7.77 (s, 1 H, H-6), 4.35 (t, J =
5.1 Hz, 4 H, 2xCH₂O), 3.88 (s, 3 H, CO₂CH₃), 3.73 (t, J = 5.1 Hz, 4 H, N(CH₂)CH₂), 3.13 (s,
20 6 H, 2xSO₂CH₃). Anal. Calcd for C₁₄H₁₉N₂O₁₂S₂: C, 34.6; H, 3.9; N, 8.7; S, 13.2. Found: C,
34.8; H, 3.7; N, 8.9; S, 13.1%.

Hydrolysis of 7 (3.0 g, 6.18 mmol) with 3 N KOH (40 mL) in dioxane (200 mL) at room
temperature for 15 min followed by acidification with 1 N HCl and extraction with EtOAc
gave a quantitative yield of 5-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-
25 dinitrobenzoic acid (8), mp 200-210 °C, which was used for the next step without further
purification; ¹H NMR [(CD₃)₂SO] δ 14.1 (v br s, 1 H, CO₂H), 8.57 (s, 1 H, H-3), 7.69 (s, 1
H, H-6), 4.34 (t, J = 5.1 Hz, 4 H, 2xCH₂O), 3.72 (t, J = 5.1 Hz, 4 H, 2xCH₂CH₂O), 3.13 (s,
6 H, 2xCH₃).

30 A suspension of 8 (3.20 g, 6.79 mmol) in SOCl₂ (60 mL) containing DMF (2 drops) was
heated under reflux for 1 h. Evaporation of the solvent under reduced pressure, followed
by azeotroping in with benzene gave the crude acid chloride, which was dissolved in dry
Me₂CO (80 mL) and treated at 0 °C with 2-aminoethanol (1.24 g, 20.3 mmol). After
stirring at 0 °C for 5 min, the mixture was acidified to pH 2-3 with 0.2 N HCl,

concentrated to half volume, and then solid NaBr was added. The mixture was extracted with EtOAc (2x) and the combined extracts were washed with saturated NaBr solution, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel, eluting with EtOAc/MeOH (15:1) to give 2-(5-[(2-hydroxyethyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**9a**) (2.87 g, 82%) as a gum that was used directly.

A mixture of **9a** (1.80 g, 3.50 mmol) and LiBr (0.43 g, 4.95 mmol) in DMF (5 mL) was stirred at 60 °C for 2 h. The reaction was then poured into saturated NaBr solution and extracted with EtOAc (2x). The combined extracts were washed with saturated NaBr solution, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc, to give 5-[bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-2,4-dinitrobenzamide (**10a**) (0.78 g, 46%): mp (MeOH/EtOAc/pet. ether) 151-152 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (t, J = 5.7 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.76 (t, J = 5.6 Hz, 1 H, OH), 3.77-3.64 (m, 8 H, $\text{N}(\text{CH}_2\text{CH}_2\text{Br})_2$), 3.53 (q, J = 6.0 Hz, 2 H, CH_2OH), 3.31 (q, partially obscured, J = 6.1 Hz, 2 H, CONHCH₂). Anal. calcd for $\text{C}_{13}\text{H}_{16}\text{Br}_2\text{N}_4\text{O}_6$: C, 32.3; H, 3.3; 11.6; 33.0. Found: C, 32.6; H, 3.3; N, 11.6; Br, 33.3%.

Further elution with EtOAc/MeOH (9:1) gave **IIa5** (0.73 g, 42%): mp (EtOAc) 102-104 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.70 (t, J = 5.7 Hz, 1 H, CONH), 8.54 (s, 1 H, H-3), 7.46 (s, 1 H, H-6), 4.76 (J = 5.5 Hz, 1 H, OH), 4.34 (t, J = 5.1 Hz, 2 H, CH_2OSO_2), 3.74 (t, J = 5.1 Hz, 2 H, $\text{CH}_2\text{CH}_2\text{OSO}_2$), 3.70 (br s, 4 H, $\text{CH}_2\text{CH}_2\text{Br}$), 3.53 (q, J = 6.0 Hz, 2 H, CH_2OH), 3.31 (q, partially obscured, J = 6.1 Hz, 2 H, CONHCH₂), 3.14 (s, 3 H, CH_3). Anal. calcd for $\text{C}_{14}\text{H}_{19}\text{BrN}_4\text{O}_9\text{S}$: C, 34.3; H, 3.9; N, 11.0; Br, 15.9. Found: C, 33.8; H, 3.8; H, 11.2; Br, 16.0%.

2-((2-Bromoethyl)-5-[(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (IIa6**). Reaction of the crude acid chloride made as above from acid **8** (2.9 g, 6.15 mmol) was dissolved in Me_2CO (100 mL), cooled in an ice-bath and treated with an excess of 3-amino-1,2-propanediol. After stirring for 10 min. the reaction mixture was acidified to pH 2-3 with 1 N HCl, most of the solvent was evaporated, and the residue was partitioned between water and EtOAc. The aqueous layer was re-extracted with EtOAc and**

the combined organic phases were dried and evaporated. The residue was adsorbed directly onto silica gel and chromatographed, elution with EtOAc/MeOH (from 50:1 to 10:1) giving 2-(5-[(2,3-dihydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**9b**) (2.92 g, 87%) as a yellow oil; ¹H NMR

5 $[(CD_3)_2SO]$ δ 8.66 (t, J = 5.8 Hz, 1 H, CONH), 8.54 (s, 1 H, H-3), 7.48 (s, 1 H, H-6), 4.81 (d, J = 5.0 Hz, 1 H, CHOH), 4.59 (t, J = 5.1 Hz, 1 H, CH₂OH), 4.35 (m, 4 H, 2x CH₂OMs), 3.66 (m, 4 H), 3.62 (m, 1 H), 3.46 – 3.36 (m, 4 H), 3.13 (s, 6 H); ¹³C NMR δ 164.48, 147.09, 138.26, 137.27, 136.60, 124.17, 121.72, 70.02, 66.69, 63.68, 50.21, 42.68, 36.55. HRMS m/z (M+1)⁺ required for C₁₆H₂₅N₄O₁₃S₂ 545.08596; Found 545.0856.

10

A solution of **9b** (1.28 g, 2.53 mmol) was dissolved in EtOAc (100 mL) and treated with LiBr (347 mg, 4.0 mmol) at 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (from 1:0 to 10:2) gave 5-[bis(2-bromoethyl)amino]-N-(2,3-dihydroxypropyl)-2,4-dinitrobenzamide (**10b**) (0.4 g, 31%) as a foam; ¹H NMR

15 $[(CD_3)_2SO]$ δ 8.71 (t, J = 5.8 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.86 (d, J = 5.0 Hz, 1 H, CHOH), 4.59 (t, J = 5.8 Hz, 1 H, CH₂OH), 3.70 – 3.10 (m, 13 H); ¹³C NMR δ 164.61, 146.65, 137.99, 137.35, 136.52, 124.25, 121.20, 70.05, 63.73, 52.44, 42.76, 30.33. HRMS m/z (M+1)⁺ required for C₁₄H₁₉⁷⁹Br₂N₄O₇ 512.9621; Found 512.9596.

20

Further elution gave **IIa6** (0.62 g, 46%): mp (EtOAc) 117-118 °C; ¹H NMR $[(CD_3)_2SO]$ δ 8.68 (t, J = 5.8 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.46 (s, 1 H, H-6), 4.82 (d, J = 5.0 Hz, 1 H, CHOH), 4.56 (t, J = 5.1, 1 H, CH₂OH), 4.32 (m, 2 H, CH₂OMs), 3.75-3.60 (m, 7 H), 3.46-3.36 (m, 4 H), 3.13 (s, 3 H); ¹³C NMR δ 164.48, 146.84, 138.05, 137.29, 136.52, 124.18, 121.40, 70.01, 66.74, 63.68, 52.89, 49.56, 42.69, 36.55, 30.20. Anal. Calcd for C₁₅H₂₁BrN₄O₁₀S: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.0; H, 4.0; N, 10.5; Br, 15.2%.

30 Further elution gave starting material (**9b**) (0.27 g, 20%).

Example B : Preparation of analogues of class IIb by the method outlined in Scheme 2.

2-[2-(Aminocarbonyl)(2-chloroethyl)-4,6-dinitroanilino]ethyl methanesulfonate

(IIb1). A solution of 2-[bis(2-hydroxyethyl)amino]-3,5-dinitrobenzamide [Friedlos et al.,

5 *J. Med. Chem.*, 1997, 40, 1270] (2.5 g, 8 mmol) in CH_2Cl_2 (200 mL) was cooled in an ice-bath and Et_3N (8 mL) and MsCl (4 mL) were added in one portion. After stirred for 10 min, satd. NaHCO_3 (100 mL) was added, and after a further 30 min the aqueous phase was extracted with CH_2Cl_2 (2x70 mL), the combined organic phase were dried, concentrated under reduced pressure, and the residue was purified by column chromatography on silica 10 gel. Elution with $\text{EtOAc}/\text{petroleum ether}$ (1:1 to 1:0), gave **IIb1** (0.6 g, 18%): mp (EtOAc/petroleum ether) 155-157 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, J = 2.7 Hz, 1 H, H-5), 8.34 (d, J = 2.7 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 7.99 (s, 1 H, CONH), 4.29 (m, 2 H, CH_2OMs), 3.73 (m, 2 H, CH_2Cl), 3.48 (m, 4 H, 2x CH_2N), 3.15 (s, 3 H, OSO_2CH_3); ^{13}C NMR δ 167.11, 145.98, 146.34, 140.84, 136.05, 127.26, 122.22, 67.49, 54.35, 51.34, 41.36, 36.46. Anal. Calcd for $\text{C}_{12}\text{H}_{15}\text{ClN}_4\text{O}_8\text{S}$: C, 35.1; H, 3.7; N, 13.7; Cl, 8.5. Found: C, 35.7; H, 3.9; N, 13.6; Cl, 8.7%. Further elution gave 2-(2-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13a**) (3.0 g, 80%): mp (EtOAc) 149-150 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (d, J = 2.8 Hz, 1 H, H-5), 8.35 (d, J = 2.9 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 8.00 (s, 1 H, CONH), 4.31 (m, 4 H, 2x CH_2OMs), 3.49 (m, 4 H, 2x $\text{CH}_2\text{-N}$), 3.14 (s, 6 H, 2x OSO_2CH_3). Anal. Calcd for $\text{C}_{13}\text{H}_{18}\text{N}_4\text{O}_{11}\text{S}_2$: C, 33.2; H, 3.9; N, 11.9. Found: C, 33.7; H, 4.0; N, 11.8%.

2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate

(IIb2). A solution of dimesylate **13a** (1.62 g, 3.5 mmol) in warm EtOAc (100 mL) was

25 treated with one portion of LiBr (400 mg, 4.7 mmol), and the mixture was heated to 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with $\text{EtOAc}/\text{petroleum ether}$ (1:1 to 1:0) gave the dibromide (0.31 g, 20%) as yellow solid. (lit., foam) [Friedlos et al., *J. Med. Chem.*, 1997, 1270]. Further elution gave **IIb2** (0.85 g, 53%): mp (EtOAc/petroleum ether) 153-154 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, J = 2.8 Hz, 1 H, H-5), 8.33 (d, J = 2.8 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 7.99 (s, 1 H, CONH), 4.29 (m, 2 H, CH_2OMs), 3.60 (m, 2 H, CH_2Br), 3.49 (m, 4 H, 2x $\text{CH}_2\text{-N}$), 3.14 (s, 3 H, OSO_2CH_3); ^{13}C NMR δ 167.11, 145.75, 146.37, 140.92, 136.12, 127.24, 122.20, 67.53, 54.41, 51.16, 36.46, 29.73. Anal. Calcd for 146.37, 140.92, 136.12, 127.24, 122.20, 67.53, 54.41, 51.16, 36.46, 29.73. Anal. Calcd for

$C_{12}H_{15}BrN_4O_8S$: C, 31.7; H, 3.3; N, 12.3; Br, 17.4. Found: C, 31.4; H, 3.4; N, 12.3; Br, 17.8%.

2-((2-Bromoethyl)-2-[(2-hydroxyethyl)amino]carbonyl)-4,6-dinitroanilino)ethyl
 5 methanesulfonate (IIb3). 2-Aminoethanol (2.9 g, 47 mmol) in 5 mL of water was added
 in one portion to a solution of crude 2-chloro-3,5-dinitrobenzoic acid chloride [prepared
 from 2-chloro-3,5-dinitrobenzoic acid 11 (5.0 g, 18.3 mmol) with $SOCl_2$] in Me_2CO (50
 mL) while cooling in an ice-bath. The mixture was stirred for 30 min, then acidified with
 10 1N HCl to pH 4-5 and concentrated under reduced pressure to remove the Me_2CO . EtOAc
 (100 mL) was added, and after 2 h a white solid was collected, washed with EtOAc and air-
 dried to give 2-chloro-3,5-dinitro-N-(2-hydroxyethyl)benzamide (12b) (3.0 g, 36%): mp
 (EtOAc) 159-160 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.99 (d, J = 2.6 Hz, 1 H, H-5), 8.86 (m, 1 H,
 CONH), 8.56 (d, J = 2.6 Hz, 1 H, H-3), 4.83 (m, 1 H, -OH), 3.54 (m, 4 H) which was used
 for next step without further purification.

15 A solution of 12b (0.6 g, 2.14 mmol) in CH_2Cl_2 was cooled in an ice-bath, and 3,4-
 dihydro-2H-pyran (2.0 mL) and p-toluenesulfonic acid (0.1 g) were added. The reaction
 mixture was stirred for 2 h, then concentrated under reduced pressure. Chromatography of
 the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-
 20 chloro-3,5-dinitro-N-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]benzamide (12c) (0.8 g,
 100%): as an oil; 1H NMR $[(CD_3)_2SO]$ δ 8.67 (d, J = 2.6 Hz, 1 H, H-4), 8.60 (d, J = 2.6
 Hz, 1 H, H-6), 7.02 (m, 1 H, CONH), 4.54 (m, 1 H), 4.00-3.50 (m, 6 H), 1.84-1.75 (m, 6
 H) which was used for next step without further purification. Reaction of 7c with
 25 diethanolamine, followed by $MsCl/Et_3N$ as described above, gave 2-[{2-
 [(methylsulfonyl)oxy]ethyl}-4,6-dinitro-6-({[2-(tetrahydro-2H-pyran-2-
 yloxy)ethyl]amino}carbonyl)anilino]ethyl methanesulfonate (13d) (1.28 g, 100%): as a
 yellow foam; 1H NMR $[(CD_3)_2SO]$ δ 8.63 (d, J = 2.9 Hz, 1 H, H-5), 8.51 (d, J = 2.9 Hz, 1
 H, H-3), 4.55 (m, 1 H), 4.39 (m, 4 H), 4.00-3.59 (m, 10 H), 3.15 (s, 3 H), 3.03 (s, 3 H),
 1.64-1.39 (m, 6 H) which was used in the next step without further purification.

30 A solution of 13d (1.28 g, 2.14 mmol) in THF (60 mL) was treated with 1 N HCl (40 mL),
 and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized
 with satd. $NaHCO_3$, and extracted with EtOAc (3x80 mL). The combined organic phases
 were washed with brine and dried, the solvent was evaporated, and the residue was

purified by chromatography on silica gel, eluting with EtOAc/MeOH (from 1:0 to 100:2), to give **13c** (0.84 g, 76%): as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.78 (m, 1 H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-5), 8.36 (d, *J* = 2.7 Hz, 1 H, H-3), 4.29 (m, 4 H, 2xCH₂OMs), 3.56 (m, 2 H), 3.45 (m, 6 H), 3.14 (s, 6 H, 2xOSO₂CH₃); ¹³C NMR δ 165.37, 146.27, 145.06, 140.63, 135.78, 127.62, 122.32, 67.26, 59.17, 51.26, 42.14, 36.44.

5 Treatment of **13c** (0.49 g, 0.95 mmol) with LiBr (0.100 g, 1.2 mmol) in EtOAc (60 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave the dibromide (**14c**) (0.24 g, 53%). Further elution gave **IIb3** (0.20 g, 42%): as yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-5), 8.36 (d, *J* = 2.7 Hz, 1 H, H-3), 4.28 (m, 2 H, CH₂OMs), 3.58 (m, 4 H), 3.44 (m, 4 H), 3.14 (s, 3 H, OSO₂CH₃); ¹³C NMR δ 165.33, 145.79, 145.20, 140.87, 135.11, 127.50, 122.19, 67.49, 59.18, 54.21, 50.99, 42.09, 36.44, 29.68. HRMS m/z (M+1)⁺ required for C₁₄H₂₀⁷⁹BrN₄O₉S 499.01344; Found 499.01324.

10

15 **2-((2-Bromoethyl)-2-{{(2,3-dihydroxypropyl)amino}carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate (IIb4).** A solution of 2-(2-({[(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino}carbonyl){2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13e**) [Palmer et al., *J. Med. Chem.* 1997, 40, 1272] (5.0 mmol) in MeOH (200 mL) was treated with p-toluenesulfonic acid (0.2 g) at room temperature for 4 h. Most of the MeOH was then evaporated, and the residue was taken up in EtOAc (200 mL), washed with satd. NaHCO₃ and brine, dried and concentrated. Chromatography of the product on silica gel, eluting with EtOAc/MeOH (20:1), gave 2-{{(2,3-dihydroxypropyl)amino}carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13d**) (2.0 g, 73%): as yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.74 (d, *J* = 3.0 Hz, 1 H, H-5), 8.37 (d, *J* = 3.0 Hz, 1 H, H-3), 4.30 (m, 4 H, 2xCH₂OMs), 3.66 (m, 1 H), 3.48-3.30 (m, 8 H), 3.14 (s, 6 H, 2xOSO₂CH₃); ¹³C NMR δ 165.42, 146.24, 145.09, 140.60, 135.77, 127.67, 122.26, 69.77, 67.29, 63.87, 51.29, 42.98, 36.44. HRMS m/z (M+1)⁺ required for C₁₆H₂₅N₄O₁₃S₂ 545.08596; Found 545.08680.

20 Treatment of **13d** (1.50 g, 2.75 mmol) with LiBr (0.21 g, 2.0 mmol) in EtOAc (60 mL) at 60 °C for 3 h, followed by chromatography on silica gel and elution with EtOAc/MeOH (20:1), gave the dibromide **9e** (0.5 g, 35%) as a yellow foam and then **IIb4** (0.62 g, 34%): yellow solid; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.8 Hz, 1 H, H-5), 8.71 (m, 1 H, CONH), 8.36 (d, *J* = 2.8 Hz, 1 H, H-3), 4.28 (m, 2 H, CH₂OMs), 3.69-3.30 (m, 11 H), 3.14 (s, 3 H);

25

30

¹³C NMR δ 165.52, 145.87, 145.30, 140.93, 136.20, 127.64, 122.23, 68.89, 67.62, 63.93, 54.35, 51.08, 43.04, 36.52, 29.80. Anal. Calcd for C₁₅H₂₁BrN₄O₁₀S: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.0; H, 4.0; N, 10.5; Br, 15.2%. Further elution gave starting material 8e (0.28, 19%).

5

2-[(2-Bromoethyl)-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate (IIb5). 2-Chloro-N-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide (12f) (0.5 g, 1.34 mmol) was reacted with diethanolamine (0.5 g) in *p*-dioxane (10 mL) at room temperature for 3 h. The reaction mixture was poured into brine, extracted with EtOAc (3x70 mL), and the combined organic phases were dried and concentrated under reduced pressure to give crude 2-[bis(2-hydroxyethyl)amino]-N-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide. This was dissolved in CH₂Cl₂ (100 mL), cooled in an ice-bath, and treated with Et₃N (1.5 mL) followed by MsCl (0.7 mL) in one portion. After stirring for 10 min, sat. NaHCO₃ (100 mL) was added and the mixture was stirred for a further 30 min, then the aqueous phase was extracted with CH₂Cl₂ (2x70 mL). The combined organic phases were dried and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with EtOAc/MeOH (20:1 to 9:0) to give yield 2-[(2-[(methylsulfonyl)oxy]ethyl)-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate (13f)

(0.75 g, 93%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-5), 8.20 (d, *J* = 2.7 Hz, 1 H, H-3), 4.28 (m, 4 H, 2x CH₂OMs), 3.56 (m, 5 H), 3.44 (m, 5 H), 3.15 (s, 6 H), 2.35 (m, 6 H), 1.71 (m, 2 H).

A solution of 8f (0.70 g, 1.17 mmol) in EtOAc (100 mL) was treated with LiBr (118 mg, 1.36 mmol) at 60 °C for 2 h. Volatiles were removed under reduced pressure, and the

residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (from 20:1 to 10:1) gave 2-[bis(2-bromoethyl)amino]-N-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide (14f) 228 mg (34%) as a yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.76 (d, *J* = 2.8 Hz, 1 H, H-5), 8.30 (d, *J* = 2.8 Hz, 1 H, H-3), 3.58-3.42 (m, 14 H), 2.36 (m, 6 H), 1.70 (m, 2 H); ¹³C NMR δ 165.08, 145.57, 145.27, 141.19, 136.40, 127.27, 122.10, 66.08, 59.66, 55.64, 53.19, 37.61, 25.39, 13.99. HRMS m/z (M+1)⁺ required for C₁₈H₂₅⁷⁹Br₂N₅O₆: 566.0250. Found: 566.0241. Later eluates gave IIb5 (300 mg, 44%); as yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.75 (d, *J* = 2.6 Hz, 1 H, H-4), 8.31 (d, *J* = 2.6 Hz, 1 H, H-6), 4.28 (m, 2 H,

CH₂OMs), 3.56 (m, 7 H), 3.44 (m, 5 H), 3.14 (s, 3 H), 2.35 (m, 6 H), 1.71 (m, 2 H); ¹³C NMR δ 165.07, 145.79, 145.31, 140.92, 136.04, 127.36, 122.21, 67.50, 66.09, 59.64, 55.68, 53.21, 51.10, 37.63, 36.45, 25.41, 14.00. HRMS m/z (M+1)⁺ required for C₁₉H₂₉⁷⁹BrN₅O₉S 582.08519. Found 582.08694; together with starting material 13f (117 mg, 18%).

Methyl 3-{{2-((2-chloroethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl}amino}propanoate (IIb6). Methyl alanine hydrochloride (2.55 g, 18.3 mmol) was dissolved in water (12 mL), and the solution was diluted with Me₂CO (20 mL) and Et₂O (50 mL). This was then poured into a solution of crude 2-chloro-3,5-dinitrobenzoyl chloride [prepared from 2-chloro-3,5-dinitrobenzoic acid 11 (5.0 g, 18.3 mmol) with SOCl₂] in Me₂CO (50 mL) while cooling in an ice-bath. The mixture was stirred for 30 min, then poured into water and extracted with EtOAc. The organic phase was washed with satd. NaHCO₃ and brine, dried, and concentrated to give methyl 3-[(2-chloro-3,5-dinitrobenzoyl)amino]propanoate (12g) (4.45 g, 73.3%): mp (EtOAc/petroleum ether) 128-130 °C; ¹H NMR [(CD₃)₂SO] δ 8.99 (d, *J* = 2.7 Hz, 1 H, H-4), 8.96 (m, 1 H, CONH), 8.51 (d, *J* = 2.7 Hz, 1 H, H-6), 3.63 (s, 3 H, CO₂CH₃), 3.50 (m, 2 H, CONHCH₂), 2.64 (m, 2 H, CH₂CO₂). The product was used without further purification. A mixture of 12g (2.5 g, 7.6 mmol) and diethanolamine (2.0 g) in *p*-dioxane (30 mL) was kept at room temperature for 3 h, then poured into brine and extracted with EtOAc (3x70 mL). The combined organic phases were dried and evaporated under reduced pressure. The residue was dissolved in CH₂Cl₂ (15 mL), cooled in an ice-bath, and treated with Et₃N (8 mL) and MsCl (4 mL). After stirring for 10 min, satd. NaHCO₃ (100 mL) was added, and following a further 30 min of stirring the aqueous phase was extracted with CH₂Cl₂ (2x70 mL). The combined organic phases were dried and then evaporated under reduced pressure, and the residue was then purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0) gave IIb6 (0.2 g, 5%): as yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.88 (m, 1 H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.31 (d, *J* = 2.7 Hz, 1 H, H-6), 4.29 (m, 2 H, CH₂OMs), 3.71 (m, 2 H, CH₂Cl), 3.63 (s, 3 H, CO₂CH₃), 3.54 – 3.36 (m, 6 H), 3.14 (s, 3 H, OSO₂CH₃), 2.65 (m, 2 H, CH₂CO₂); ¹³C NMR δ 171.68, 165.34, 146.14, 145.17, 140.74, 135.59, 127.58, 122.42, 67.47, 54.22, 51.45, 51.22, 41.37, 36.48, 35.44, 32.95. HRMS m/z (M+1)⁺ required for C₁₆H₂₂³⁵ClN₄O₁₀S; 497.0745. Found; 497.0748.

Further elution gave methyl 3-{{2-[{(methylsulfonyl)oxy]ethyl}amino]-3,5-dinitrobenzoyl}amino}propanoate (**13g**) (2.6 g, 62%): as yellow oil; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.90 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.32 (d, $J = 2.7$ Hz, 1 H, H-6), 4.30 (m, 4 H, 2xCH₂OMs), 3.63 (s, 3 H, CO₂CH₃), 3.52 (m, 2 H, CONHCH₂), 3.44 (m, 4 H, 2xCH₂N), 3.14 (s, 6 H, 2xOSO₂CH₃), 2.65 (m, 2 H, CH₂CO₂); ^{13}C NMR δ 171.66, 165.28, 146.36, 144.98, 140.52, 135.23, 127.64, 122.50, 67.20, 51.40, 51.25, 36.44, 35.45, 32.91. HRMS m/z (M+1)⁺ required for C₁₇H₂₅N₄O₁₃S₂: 557.0860. Found: 557.0853.

In an alternative preparation of **IIb6**, a solution of **13g** (0.417 g, 0.75 mmol) in DMF (10 mL) was treated with LiCl (0.038 g, 1.00 mmol) at 60 °C for 2 h, and then cooled and 10 poured into dilute HCl and extracted with EtOAc (3x80 mL). Workup and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether from 1:1 to 2:1, gave methyl 3-{{2-[bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl}amino}propanoate (**15g**) (0.16 g, 51%): as yellow oil; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.85 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.29 (d, $J = 2.7$ Hz, 1 H, H-6), 3.68 (m, 4 H, 2xCH₂Cl), 3.63 (s, 3 H, CO₂CH₃), 3.50 (m, 2 H, CONHCH₂), 3.41 (m, 4 H, N(CH₂)₂), 2.64 (m, 2 H, CH₂CO₂); ^{13}C NMR δ 171.59, 165.28, 145.81, 145.31, 140.89, 135.89, 127.45, 122.26, 54.08, 51.40, 41.51, 35.35, 32.92. . Further elution then gave **IIb6** (0.124 g, 33%), identical with the sample prepared above.

20 **Methyl 3-{{2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl}amino}propanoate (IIb7).** Treatment of **13g** (2.04 g, 3.67 mmol) with LiBr (0.318 g, 3.67 mmol) in EtOAc (100 mL) at 60 °C for 3 h, followed by chromatography on silica gel and elution with EtOAc/petroleum ether from 1:1 to 1:0 gave methyl 3-{{2-[bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl}amino}propanoate (**14g**) (0.55 g, 29%): as yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.86 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.29 (d, $J = 2.7$ Hz, 1 H, H-6), 3.63 (s, 3 H, CO₂CH₃), 3.60 – 3.43 (m, 10 H), 2.64 (m, 2 H, CH₂CO₂); ^{13}C NMR δ 171.60, 165.28, 145.39, 145.36, 141.07, 136.05, 127.44, 122.25, 53.97, 51.44, 35.35, 32.95, 29.96. HRMS m/z (M+1)⁺ required for C₁₅H₁₉⁷⁹Br₂N₄O₇: 524.9621. Found: 524.9616.

25 Further elution gave **IIb7** (0.96 g, 48%): as yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.89 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.31 (d, $J = 2.7$ Hz, 1 H, H-6), 4.28 (m, 2 H, CH₂OMs), 3.63 (s, 3 H, CO₂CH₃), 3.60 – 3.43 (m, 8 H), 3.14 (s, 3 H, OSO₂CH₃), 2.65 (m, 2 H, CH₂CO₂); ^{13}C NMR δ 171.63, 165.28, 145.87, 145.19, 140.81, 135.65, 127.54,

122.37, 67.47, 54.25, 51.42, 51.02, 36.45, 35.40, 32.93, 29.69. HRMS m/z (M+1)⁺ required for C₁₆H₂₂⁷⁹BrN₄O₁₀S: 541.0240. Found: 541.0228, followed by starting material 13g (0.45 g, 22%).

5

Example C : Preparation of analogues of class IIc by the method outlined in Scheme 3.

2-[3-(Aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc1).

10 A solution of methyl 3-[bis(2-hydroxyethyl)amino]-2,6-dinitrobenzoate [Palmer et al., *J. Med. Chem.* 1996, 39, 2518] (7.24 g, 22 mmol) in CH₂Cl₂ (120 mL) was cooled in an ice-bath and Et₃N (15 mL) and MsCl (8 mL) were added in one portion. After stirred for 10 min, satd. NaHCO₃ (100 mL) was added, and after a further 30 min the aqueous phase was extracted with CH₂Cl₂ (2x70 mL), the combined organic phase were dried, concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0), gave crude methyl 3-[bis{2-[(methylsulfonyl)oxy]ethyl}amino]-2,6-dinitrobenzoate (16) (10.67 g, 100%) as a yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.32 (d, *J* = 9.6 Hz, 1 H, H-5), 7.75 (d, *J* = 9.6 Hz, 1 H, H-4), 4.32 (m, 4 H), 3.88 (s, 3 H), 3.67 (m, 4 H), 3.14 (m, 6 H); ¹³C NMR δ 163.02, 147.59, 138.40, 136.46, 128.33, 125.83, 123.96, 66.73, 54.00, 50.24, 45.58, 36.58.

Hydrolysis of 16 (10.6 g, 21.9 mmol) with 3 N KOH (40 mL) in dioxane (200 mL) at room temperature for 15 min, followed by acidification with 1 N HCl and extraction with EtOAc, gave a quantitative yield of crude 3-[bis{2-[(methylsulfonyl)oxy]ethyl}amino]-2,6-dinitrobenzoic acid (17): mp 200-210 °C. HRMS: C₁₃H₁₈N₃O₁₂S₂ requires m/z 472.0332. Found: 472.033, that was used without purification. The acid chloride (SOCl₂/cat. DMF) from 17 (3.2 g, 6.8 mmol) was dissolved in Me₂CO (30 mL), cooled in an ice-bath and treated with concentrated NH₄OH (10 mL). After stirring for 10 min. the reaction mixture was acidified to pH 2-3 with 1 N HCl, then most of the solvent was evaporated and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (2x80 mL) and the combined organic extracts were dried and evaporated under reduced pressure. The residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave IIc1 (0.145

g, 5.2%: mp (EtOAc) 134-136 °C; ¹H NMR [(CD₃)₂SO] δ 8.25 (d, *J* = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.64 (d, *J* = 9.3 Hz, 1 H, H-6), 4.27 (m, 2 H, CH₂OMs), 3.73 (m, 2 H), 3.66 (m, 2 H), 3.59 (m, 2 H), 3.15 (s, 3 H); ¹³C NMR δ 163.06, 146.40, 140.52, 137.65, 129.42, 127.51, 122.89, 66.83, 52.93, 50.16, 41.45, 36.57. Anal. Calcd. For C₁₂H₁₅ClN₄O₈S: C, 35.1; H, 3.7; N, 13.6; Cl, 8.6. Found: C, 35.5; H, 3.7; N, 13.6; Cl, 8.6%.

Elution of the column with EtOAc/MeOH (50:1) gave 2-(3-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,6-dinitroanilino)ethyl methanesulfonate (**18a**) (1.1 g, 34%): mp (EtOAc/MeOH/petroleum ether) 160-162 °C; ¹H NMR [(CD₃)₂SO] δ 8.26 (d, *J* = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.66 (d, *J* = 9.3 Hz, 1 H, H-6), 4.27 (m, 4 H, 2xCH₂OMs), 3.63 (m, 4 H, 2xCH₂N), 3.15 (s, 6 H, 2xCH₃SO₃⁻); ¹³C NMR δ 163.00, 146.51, 140.98, 137.99, 129.30, 127.47, 123.40, 66.74, 50.44, 36.56. Anal. Calcd. For C₁₃H₁₈N₄O₁₁S₂: C, 33.2; H, 3.9; N, 11.9. Found: C, 33.5; H, 3.8; N, 11.9%.

15 2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate (IIc2). LiBr (117 mg, 1.34 mmol) was added in one portion to a solution of **18a** (0.474 g, 1.0 mmol) in Me₂CO/EtOAc (1:1, 100 mL), and the reaction mixture was heated to 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (1:1) gave 3-[bis(2-bromoethyl)amino]-2,6-dinitrobenzamide (**19a**) (95 mg, 21%): as a yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.25 (d, *J* = 9.5 Hz, 1 H, H-5), 8.22 (s, 1 H, NH), 7.88 (s, 1 H, NH), 7.63 (d, *J* = 9.5 Hz, 1 H, H-4), 3.68 (m, 4 H), 3.58 (m, 4 H) (Lit. [Palmer et al., J. Med. Chem., 1996, 39, 2518-2528].

Further elution with EtOAc/petroleum ether (3:1) gave **IIc2** (208 mg, 46%): mp (EtOAc/petroleum ether) 143-145 °C; ¹H NMR [(CD₃)₂SO] δ 8.25 (d, *J* = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.64 (d, *J* = 9.3 Hz, 1 H, H-6), 4.28 (m, 2 H, CH₂OMs), 3.67 (m, 4 H), 3.57 (m, 2 H), 3.16 (s, 3 H); ¹³C NMR δ 163.05, 146.17, 140.49, 137.68, 129.42, 127.53, 122.89, 66.85, 52.92, 50.04, 36.57, 29.95. Anal. Calcd. For C₁₂H₁₅BrN₄O₈S: C, 31.7; H, 3.3; N, 12.3; Br, 17.4. Found: C, 31.9; H, 3.3; N, 12.2; Br, 17.5%.

Later eluates gave starting material **18a** (150 mg).

2-((2-Chloroethyl)-3-[(3-hydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (**IIc3**). Reaction of the acid chloride of **17** with 3-aminopropanol in Me₂CO at 0 °C as described above, followed by chromatography of the product on silica gel and elution with EtOAc/petroleum ether (1:1), gave **IIc3** (292 mg, 12%): mp 5 (EtOAc/petroleum ether) 104-109 °C; ¹H NMR [(CD₃)₂SO] δ 8.75 (t, *J* = 5.8 Hz, 1 H, CONH), 8.24 (d, *J* = 9.4 Hz, 1 H, H-5), 7.64 (d, *J* = 9.4 Hz, 1 H, H-6), 4.44 (m, 1 H, CHOH), 4.26 (m, 2 H), 3.72 (m, 2 H), 3.65 (m, 2 H), 3.59 (m, 2 H), 3.43 (m, 2 H), 3.20 (m, 2 H), 3.15 (s, 3 H), 1.60 (m, 2 H); ¹³C NMR δ 161.09, 146.42, 140.49, 137.65, 129.23, 127.58, 122.91, 66.82, 58.22, 52.88, 50.11, 41.44, 36.57, 36.37, 31.57. Anal. Calcd. For C₁₅H₂₁ClN₄O₉S: C, 38.5; H, 4.5; N, 12.0; Cl, 7.5. Found: C, 38.8; H, 4.8; N, 11.5; Cl, 7.0%.

10 Further elution with EtOAc gave 2-(3-[(3-hydroxypropyl)amino]carbonyl)-{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**18b**) (1.1 g, 41%): mp (EtOAc/MeOH/petroleum ether) 160-162 °C; ¹H NMR [(CD₃)₂SO] δ 8.77 (t, *J* = 5.8 Hz, 1 H, CONH), 8.26 (d, *J* = 9.4 Hz, 1 H, H-5), 7.66 (d, *J* = 9.4 Hz, 1 H, H-6), 4.43 (m, 1 H, CHOH), 4.27 (m, 4 H, 2xCH₂OMs), 3.63 (m, 4 H, 2xCH₂N), 3.43 (m, 2 H), 3.20 (m, 2 H), 3.15 (s, 6 H, 2xCH₃SO₃), 1.60 (m, 2 H); ¹³C NMR δ 161.03, 146.52, 140.95, 138.00, 129.12, 127.54, 123.42, 66.72, 58.22, 50.39, 36.55, 36.37, 31.57. Anal. Calcd. For C₁₆H₂₄N₄O₁₂S₂: C, 36.4; H, 4.6; N, 10.6. Found: C, 36.6; H, 4.5; N, 10.6%.

20 2-((2-Bromoethyl)-3-[(3-hydroxypropyl)amino]carbonyl)-2,6-dinitroanilino)ethyl methanesulfonate (**IIc4**). Treatment of **18b** (716 mg, 1.36 mmol) in EtOAc (200 mL) with LiBr ((175 mg, 2.0 mmol) as above, followed by chromatography on silica gel and elution with EtOAc/ petroleum ether (from 1:1 to 1:0) gave 3-[bis(2-bromoethyl)amino]-*N*-(3-hydroxypropyl)-2,6-dinitrobenzamide (**19b**) (289 mg, 42%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.75 (t, *J* = 5.8 Hz, 1 H, CONH), 8.23 (d, *J* = 9.4 Hz, 1 H, H-5), 7.62 (d, *J* = 9.4 Hz, 1 H, H-4), 4.47 (m, 1 H, CHOH), 3.68 (m, 4 H), 3.57 (m, 4 H), 3.43 (m, 2 H), 3.20 (m, 2 H), 1.60 (m, 2 H); ¹³C NMR δ 161.20, 146.90, 140.20, 137.53, 129.36, 127.69, 122.56, 58.29, 52.64, 36.42, 31.61, 30.13. HRMS m/z (M+1)⁺ required for C₁₄H₁₉⁷⁹Br₂N₄O₆: 496.9671. Found: 496.9667.

25 Further elution with EtOAc/MeOH (50:2) gave **IIc4** (270 mg, 39%): mp. 115-117 °C; ¹H NMR [(CD₃)₂SO] δ 8.75 (t, *J* = 5.8 Hz, 1 H, CONH), 8.24 (d, *J* = 9.4 Hz, 1 H, H-5), 7.64 (d, *J* = 9.4 Hz, 1 H, H-6), 4.43 (m, 1 H, CHOH), 4.27 (m, 2 H, CH₂OMs), 3.66 (m, 4 H, 2xCH₂N), 3.59 (m, 2 H), 3.44 (m, 2 H), 3.22 (m, 2 H), 3.15 (s, 3 H, CH₃SO₃), 1.60 (m, 2

H); ^{13}C NMR δ 161.08, 146.19, 140.47, 137.69, 129.24, 127.59, 122.91, 66.83, 58.22, 52.87, 50.00, 36.57, 36.37, 31.58, 29.95. Anal. Calcd. For $\text{C}_{15}\text{H}_{21}\text{BrN}_4\text{O}_9\text{S}$: C, 35.2; H, 4.1; N, 10.9; Br, 15.4. Found: C, 35.4; H, 3.9; N, 11.0; Br, 16.3%.

5 **2-((2-Chloroethyl)-3-[(2,3-dihydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (IIc5).** Reaction of the acid chloride of **17** (2.4 g, 5.1 mmol) with 3-amino-1,2-propanediol Me_2CO at 0 °C as described above, followed by chromatography of the product on silica gel and elution with EtOAc , gave **IIc5** (240 mg, 10%): mp (EtOAc) 100-105 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.77 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.64 (d, J = 9.4 Hz, 1 H, H-6), 4.72 (d, J = 4.9, 1 H, CHO H), 4.52 (t, J = 5.7, 1 H, CH_2OH), 4.27 (m, 2 H, CH_2OMs), 3.74 – 3.50 (m, 10 H), 3.15 (s, 3 H, CH_3SO_3), 3.04 (m, 1 H); ^{13}C NMR δ 161.48, 146.38, 140.55, 137.73, 129.28, 127.51, 122.88, 69.89, 66.83, 63.57, 52.95, 50.17, 42.55, 41.43, 36.58. Anal. Calcd. For $\text{C}_{15}\text{H}_{21}\text{ClN}_4\text{O}_{10}\text{S}$: C, 37.2; H, 4.4; N, 11.6; Cl, 7.2. Found: C, 38.0; H, 4.5; N, 11.1; Cl, 7.2%.

10 Further elution with EtOAc/MeOH (50 : 1) gave **2-(3-[(2,3-dihydroxypropyl)amino]carbonyl)-2-[(methylsulfonyloxy)ethyl]-2,4-dinitroanilino)ethyl methanesulfonate (18c)** (480 mg, 51%): mp (MeOH/EtOAc) 60-63 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.78 (t, J = 5.8 Hz, 1 H, CONH), 8.24 (d, J = 9.4 Hz, 1 H, H-5), 7.66 (d, J = 9.4 Hz, 1 H, H-6), 4.72 (d, J = 4.9, 1 H, CHO H), 4.52 (t, J = 5.7, 1 H, CH_2OH), 4.27 (m, 4 H, 2x CH_2OMs), 3.63 (m, 4 H), 3.52 – 3.30 (m, 5 H), 3.15 (s, 3 H, 2x CH_3SO_3), 3.06 (m, 1 H); ^{13}C NMR δ 161.43, 146.49, 141.01, 138.07, 129.15, 127.47, 123.36, 69.89, 66.73, 63.67, 50.44, 42.55, 36.56. Anal. Calcd. For $\text{C}_{16}\text{H}_{24}\text{N}_4\text{O}_{13}\text{S}_2$: C, 35.3; H, 4.5; N, 10.3. Found: C, 35.8; H, 4.5; N, 10.5%..

15 **2-((2-Bromoethyl)-3-[(2,3-dihydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (IIc6).** Treatment of **18c** (0.92 g, 1.7 mmol) in EtOAc (200 mL) with LiBr (170 mg, 1.95 mmol) as above, followed by chromatography on silica gel and elution with EtOAc/MeOH (50:1), gave **3-[bis(2-bromoethyl)amino]-N-(2,3-dihydroxypropyl)-2,4-dinitrobenzamide (19c)** (155 mg, 18%) as yellow oil; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.76 (t, J = 5.8 Hz, 1 H, CONH), 8.23 (d, J = 9.5 Hz, 1 H, H-5), 7.63 (d, J = 9.5 Hz, 1 H, H-6), 4.72 (d, J = 5.1 Hz, 1 H, CHO H), 4.52 (t, J = 5.7 Hz, 1 H, CH_2OH), 3.70 – 3.50 (m, 11 H), 3.04 (m, 1 H). HRMS m/z ($\text{M}+1$) $^+$ required for $\text{C}_{14}\text{H}_{19}\text{Br}_2\text{N}_4\text{O}_7$: 512.9621. Found: 512.9603.

Further elution gave **IIc6** (278 mg, 31%): mp (EtOAc) 108-110 °C; ¹H NMR [(CD₃)₂SO] δ 8.77 (t, *J* = 5.8 Hz, 1 H, CONH), 8.24 (d, *J* = 9.4 Hz, 1 H, H-5), 7.64 (d, *J* = 9.4 Hz, 1 H, H-6), 4.72 (d, *J* = 4.9, 1 H, CHO_H), 4.52 (t, *J* = 5.7, 1 H, CH₂OH), 4.27 (m, 2 H, CH₂OMs), 3.70 – 3.50 (m, 10 H), 3.15 (s, 3 H, CH₃SO₃), 3.06 (m, 1 H); ¹³C NMR δ 161.47, 146.16, 140.52, 137.77, 129.28, 127.53, 122.88, 69.89, 66.84, 63.57, 52.94, 50.05, 42.55, 36.58, 29.94. Anal. Calcd. For C₁₅H₂₁BrN₄O₁₀S: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.3; H, 4.1; N, 10.4; Br, 15.4%.

And starting material (200 mg, 22%)

10 **2-[(2-Chloroethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc7).** Reaction of the acid chloride from **17** (1.3 g) in Me₂CO with 3-(4-morpholinyl)propylamine (1.0 mL) at 0°C as described above, followed by chromatography of the product on silica gel and elution with EtOAc/MeOH (9:1 to 4:1), gave **IIc7** (0.37 g, 25%): mp (EtOAc/petroleum ether) 113-116 °C; ¹H NMR [(CD₃)₂SO] δ 8.79 (t, *J* = 5.6 Hz, 1 H, CONH), 8.25 (d, *J* = 9.4 Hz, 1 H, H-5), 7.65 (d, *J* = 9.4 Hz, 1 H, H-6), 4.28 (t, *J* = 5.3, 2 H), 3.73 (t, *J* = 6.3, 2 H), 3.66 (t, *J* = 5.2, 2 H), 3.60 (t, *J* = 5.9, 2 H), 3.56 (m, 4H), 3.17 (m, 5 H), 2.34 (m, 6H), 1.61 (m, 2H); ¹³C NMR δ 161.07, 146.44, 140.44, 137.62, 129.23, 127.60, 122.92, 66.81, 66.12, 55.40, 53.19, 52.85, 50.10, 41.45, 37.30, 36.56, 25.12. HRMS m/z (M+1)⁺ requires C₁₉H₂₉³⁵ClN₅O₉S: 538.13745. Found: 538.13869.

Later eluates gave 2-[{2-[(methylsulfonyl)oxy]ethyl}-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate (**18d**) (0.93 g, 56%) as a yellow solid, mp (EtOAc/petroleum ether) 90-95 °C; ¹H NMR [(CD₃)₂SO] δ 8.79 (t, *J* = 5.7 Hz, 1 H, CONH), 8.25 (d, *J* = 9.4 Hz, 1 H, H-5), 7.65 (d, *J* = 9.4 Hz, 1 H, H-6), 4.28 (t, *J* = 5.3, 4 H), 3.64 (t, *J* = 5.2, 4 H), 3.55 (t, *J* = 4.6, 4 H), 3.15 (m, 8 H), 2.34 (m, 6 H), 1.61 (m, 2 H); ¹³C NMR δ 161.03, 146.55, 140.90, 137.97, 129.10, 127.56, 123.43, 66.72, 66.12, 55.39, 53.19, 50.37, 37.29, 36.55, 25.13. HRMS m/z (M+1)⁺ requires C₂₀H₃₂N₅O₁₂S₂: 598.14889. Found: 598.14894.

30 **2-[(2-Bromoethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc8).** LiBr (107 mg, 1.3 mmol) was added in one portion to a warm solution of **18d** (0.53 g, 0.89 mmol) in EtOAc (50 mL). The reaction mixture was heated to 60 °C for 2 h, then volatiles were removed under reduced

pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (10:1 to 5:1) gave 3-[bis(2-bromoethyl)amino]-N-[3-(4-morpholinyl)propyl]-2,6-dinitrobenzamide (**19d**) (109 mg, 22%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (t, *J* = 5.6 Hz, 1 H, CONH), 8.23 (d, *J* = 9.4 Hz, 1 H, H-5), 7.63 (d, *J* = 9.4 Hz, 1 H, H-6), 3.68 (m, 4H), 3.57 (m, 8 H), 3.17 (m, 2 H), 2.34 (m, 6 H), 1.61 (m, 2 H). HRMS: C₁₅H₁₁⁷⁹Br₂N₄O₅ requires *m/z* 438.9253. Found: 438.9228.

5 Later eluates gave **IIc8** (293 mg, 57%): mp (EtOAc/petroleum ether) 114-117 °C; ¹H NMR [(CD₃)₂SO] δ 8.79 (t, *J* = 5.6 Hz, 1 H, CONH), 8.25 (d, *J* = 9.4 Hz, 1 H, H-5), 7.65 (d, *J* = 9.4 Hz, 1 H, H-6), 4.28 (t, *J* = 5.2, 2 H), 3.66 (m, *J* = 5.2, 4H), 3.56 (m, *J* = 4.6, 6 H), 3.17 (m, 5 H), 2.34 (m, 6 H), 1.61 (m, 2 H); ¹³C NMR δ 161.07, 146.22, 140.39, 137.65, 129.21, 127.62, 122.92, 66.83, 66.07, 55.37, 53.15, 52.83, 49.99, 37.28, 36.57, 29.97, 25.07. HRMS *m/z* (M+1)⁺ requires C₁₉H₂₉⁷⁹BrN₅O₉S: 582.08694. Found: 582.08639.

Later eluates gave starting material **18d** (124 mg, 23%).

The following Table 2 gives biological data for the compounds listed in Table 1.

Table 2. Biological data for the compounds of Table 1.

No	Human ovarian ^a			Human colon ^b		
	IC ₅₀ ^d		Ratio ^e	IC ₅₀ ^d		Ratio ^e
	NR-	NR+		NR-	NR+	
<i>Examples of formulae IIa</i>						
IIa2	226	0.84	280	150	0.69	235
IIa3	135	0.19	715	80	0.22	387
IIa4	97	0.33	311	70	0.41	172
IIa5	1110	1.74	641	804	3.0	272
<i>Examples of formulae IIb</i>						
IIb1	80	0.04	1890	20	0.07	303
IIb2	6.0	0.007	762	4.3	0.02	227
IIb3	5.2	0.04	142	3.7	0.06	66
IIb4	26	0.19	140	11	0.21	52
IIb5	3.1	0.03	102	0.89	0.05	22
IIb6	9.7	0.19	51	4.3	0.36	13
IIb7	3.7	0.15	25	1.44	0.24	6.3
<i>Examples of formulae IIc</i>						
IIc1	196	0.55	390	121	1.0	120
IIc2	150	0.21	724	85	0.31	271
IIc3	800	1.6	549	392	2.6	215
IIc4	280	0.57	497	209	0.85	301
IIc5	1680	6.6	267	856	4.3	262
IIc6	890	1.8	509	214	1.5	141
IIc7	433	32	14	262	31	8.3
IIc8	156	11	14	94	11	8.2

5 ^aHuman ovarian: wild-type (NR-) is SKOV3, transfected (NR+) is SC3.2.

^bHuman colon: wild-type (NR-) is WIDR, transfected (NR+) is WC14.10.

^cChinese hamster fibroblast: wild-type (NR-) is T-78-1, transfected (NR+) is T79-A3.

^dIC₅₀: the concentration of drug (in micromolar) required to reduce cell numbers to 50% of controls at the end of the evaluation period. ^eRatio = IC₅₀(NR-)/IC₅₀(NR+).

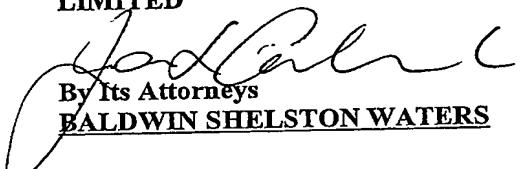
It is clear from the data of Table 2 that the examples of the nitroaniline derivatives of the invention listed in Table 2 include compounds which are active as cytotoxic agents, and which have the additional capability of being reductively activated by the *E. coli* NTR.

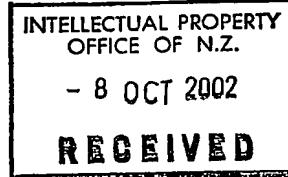
5 Wherein the foregoing description reference has been made to reagents, or integers having known equivalents thereof, then those equivalents are herein incorporated as if individually set forth.

10 While this invention has been described with reference to certain embodiments and examples, it is to be appreciated that further modifications and variations may be made to embodiments and examples without departing from the spirit or scope of the invention.

15

AUCKLAND UNISERVICES
LIMITED


By Its Attorneys
BALDWIN SHELSTON WATERS



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.